

Research Article

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


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Molecular evidence of three closely related species of *Biacetabulum* Hunter, 1927 (Cestoda: Caryophyllidea): a case of recent speciation in different fish hosts (Catostomidae)?

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Abstract

Monozoic tapeworms (Caryophyllidea) are dominant components of parasite communities of suckers (Catostomidae) in North America, with *Biacetabulum* Hunter, 1927 representing one of the more species-rich genera. Molecular (28S rDNA) and morphological (including scanning electron microscopy and histology) evaluation of newly collected tapeworms from different fish hosts revealed the existence of four similar (and three closely related) species of *Biacetabulum*. These four species differ from their congeners by having a long body (up to 48 mm long) with a very long, slender neck (its length represents $\geq 30\%$ of total body length), a large, globular scolex with a prominent central acetabulum-like loculus on the dorsal and ventral sides, two pairs of shallow lateral loculi and a distinct, slightly convex apical disc, and a cirrus-sac that is situated between the anterior arms of the ovarian wings. Taken together, the morphological and molecular data and the host associations of these species provide evidence of their host specificity. *Biacetabulum isaureae* n. sp. occurs in notch clip redhorse, *Moxostoma collapsum*, in South Carolina (USA), *B. longicollum* n. sp. in silver redhorse, *Moxostoma anisurum* (type host), and golden redhorse, *M. erythrurum*, in Manitoba (Canada) and West Virginia (USA), *B. overstreeti* n. sp. in a spotted sucker, *Minytrema melanops*, in Mississippi, and *B. hypentelii* n. sp. in northern hogsucker, *Hypentelium nigricans*, in Tennessee (USA). The new species differ from each other in the number of postovarian vitelline follicles, the posterior extent of preovarian vitelline follicles and relative size of the cirrus sac.

Introduction

Recent efforts to better understand the diversity of the parasites of freshwater fishes in North America have revealed considerable gaps in our knowledge and the existence of taxonomic problems in many groups, including caryophyllidean tapeworms (Scholz and Choudhury, 2014; Scholz and Oros, 2017). These tapeworms are a dominant group of endoparasitic helminths in catostomid fishes (Cypriniformes: Catostomidae), with about 60 described species in 22 genera (Scholz and Kuchta, 2017; Kuchta *et al.*, 2020).

To provide a robust taxonomic baseline for future ecological, evolutionary and biogeographical studies, individual genera of North American caryophyllideans are being revised, based on a critical examination of type material and newly collected and properly processed specimens, including hologenophores, from North American catostomids (Scholz *et al.*, 2015; Oros *et al.*, 2016, 2018). One of the more species-rich genera of caryophyllideans is *Biacetabulum* Hunter, 1927 with 10 recognised species (Scholz and Oros, 2017). This genus was erected by Hunter (1927) based on *B. infrequens* from the silver redhorse, *Moxostoma anisurum* (Rafinesque), from the Rock River in Illinois, USA, and at the time, as its type and only species.

None of the earlier molecular phylogenetic studies included species of *Biacetabulum* (see, e.g., Olson *et al.*, 2008; Brabec *et al.*, 2012; Scholz *et al.*, 2015). More recently, Scholz *et al.* (2021) provided sequences of two nuclear and one mitochondrial gene for several taxa of *Biacetabulum*, but did not identify most of them to species level or did so only tentatively. Therefore, a taxonomic revision of *Biacetabulum* is pending because many species are known only from original, often very brief and insufficient, descriptions (Hunter, 1927, 1929). Species composition of the genus, its monophyly and host associations of its species should also be critically assessed using molecular data.

Over the last decade, specimens of caryophyllidean tapeworms fixed using a standardized method (see Oros *et al.*, 2010) were freshly collected from a variety of fish hosts, including suckers (Catostomidae), in Canada and the USA. This new material has enabled us to critically examine the validity of several genera of North American caryophyllideans (Scholz *et al.*, 2015; Oros *et al.*, 2016, 2018). Furthermore, molecular data were generated for specimens of

Table 1. Host, geographical origin and GenBank accession data for taxa included in the phylogenetic analyses

Species	Host	Locality	Morphological voucher	GenBank ID	Source
<i>Biacetabulum isaureae</i> n. sp.	<i>Moxostoma collapsum</i>	South Carolina, USA	US 274b	MZ031042	Present study
<i>B. isaureae</i> n. sp.	<i>M. collapsum</i>	South Carolina, USA	US 272b	MZ031043	Present study
<i>B. isaureae</i> n. sp.	<i>M. collapsum</i>	South Carolina, USA	US 272c	MZ031044	Present study
<i>B. isaureae</i> n. sp.	<i>M. collapsum</i>	South Carolina, USA	US 274c/PBI-469	MW027500	Scholz <i>et al.</i> (2021)
<i>B. isaureae</i> n. sp.	<i>M. collapsum</i>	South Carolina, USA	US 275c/PBI-420	MW027501	Scholz <i>et al.</i> (2021)
<i>B. longicollum</i> n. sp.	<i>Moxostoma erythrurum</i>	Manitoba, Canada	CA36a	MZ031045	Present study
<i>B. longicollum</i> n. sp.	<i>Moxostoma anisurum</i>	Manitoba, Canada	CA38c	MZ031046	Present study
<i>B. longicollum</i> n. sp.	<i>M. erythrurum</i>	Manitoba, Canada	CA36b	MZ031047	Present study
<i>B. longicollum</i> n. sp.	<i>M. anisurum</i>	Manitoba, Canada	CA38d	MZ031048	Present study
<i>B. longicollum</i> n. sp.	<i>M. erythrurum</i>	West Virginia, USA	US 979	MZ031049	Present study
<i>B. macrocephalum</i>	<i>Catostomus commersonii</i>	Wisconsin, USA	DP66/09b	MW027498	Scholz <i>et al.</i> (2021)
<i>B. overstreeti</i> n. sp.	<i>Minytrema melanops</i>	Mississippi, USA	US 839a	MZ031050	Present study
<i>B. overstreeti</i> n. sp.	<i>M. melanops</i>	Mississippi, USA	US 209d	MZ031051	Present study
<i>B. overstreeti</i> n. sp.	<i>M. melanops</i>	Mississippi, USA	US 233b/PBI-416	MW027499	Scholz <i>et al.</i> (2021)
<i>Biacetabulum</i> sp. 1	<i>M. melanops</i>	Florida, USA	US 164a/PBI-418	MW027495	Scholz <i>et al.</i> (2021)
<i>Biacetabulum</i> sp. 2	<i>M. melanops</i>	Mississippi, USA	US 217b/PBI-415	MW027496	Scholz <i>et al.</i> (2021)
<i>Biacetabulum</i> sp. 2	<i>M. poecilurum</i>	Mississippi, USA	US 185a/PBI-465	MW027497	Scholz <i>et al.</i> (2021)
<i>Archigetes sieboldi</i>	<i>Gnathopogon elongatus</i>	Japan	–	EU343736	Olson <i>et al.</i> (2008)
<i>Archigetes</i> sp. 1	<i>Ictiobus bubalus</i>	Mississippi, USA	US 260d/PBI-464	MW027502	Scholz <i>et al.</i> (2021)
<i>Archigetes</i> sp. 2	<i>Ictiobus cyprinellus</i>	Mississippi, USA	US 242a/PBI-462	MW027493	Scholz <i>et al.</i> (2021)
<i>Archigetes</i> sp. 2	<i>Ictiobus niger</i>	Mississippi, USA	US 244b/PBI-417	MW027494	Scholz <i>et al.</i> (2021)
<i>Hunterella nodulosa</i>	<i>Catostomus commersonii</i>	USA	TS 08/53	JQ034127	Brabec <i>et al.</i> (2012)

Biacetabulum from different species of redhorses (*Moxostoma* spp.) and spotted sucker (*Minytrema melanops* (Rafinesque)). These data revealed the existence of three genetically distinct, morphologically similar, and closely related lineages of tapeworms with a conspicuously long neck. In addition, examination of specimens from *Hypentelium nigricans* (Lesueur), has shown they also represent a new species, although no ethanol-preserved material was available for molecular analyses. These four new species are described to document this case of diversification of similar and closely related tapeworms in different, often congeneric fish hosts.

Materials and methods

Molecular study

The following sequence data (28S rDNA) were used to assess phylogenetic relationships of the three newly described species of the long-necked *Biacetabulum*-species complex: (i) two unpublished 28S rRNA gene sequences of specimens from *Moxostoma collapsum* (Cope) (US 274b), South Carolina, and *M. melanops* (US 209d), Mississippi; the sequences were obtained by Alec Perkins (USA) during his internship at the Institute of Parasitology (Czech Republic) in 2016, following the protocol described by Brabec *et al.* (2012); and (ii) eight new 28S rDNA sequences (see Table 1) as follows: Genomic DNA was isolated using the E.Z.N.A. Tissue DNA Kit (Omega Bio-tek, Inc., Norcross, USA) following the manufacturer's instructions. A 1350 nucleotide (nt) long fragment of the 28S rRNA gene (D1–D3 regions) was amplified by polymerase chain reaction (PCR) following the protocol described

by Brabec *et al.* (2012). PCR amplicons were purified using exonuclease I and shrimp alkaline phosphatase enzymes (Werle *et al.*, 1994) and sequenced from both strands using the PCR primers and additional internal sequencing primer 300F (Littlewood *et al.*, 2000). Sequences were assembled and edited using Geneious version 11 (Biomatters, Auckland, New Zealand).

Novel 28S rDNA sequences of species of the *Biacetabulum*-species complex was aligned with those previously generated from species of this genus and of the genus *Archigetes* Leuckart, 1878 (Table 1) using MUSCLE (Edgar, 2004) as implemented in Geneious vs 11. The alignment was trimmed to match the shortest sequence and ambiguously aligned positions were manually excluded. The final alignment that was used for the analyses (after trimming and excluding gaps) was 1326 nucleotides long. A sequence of *Hunterella nodulosa* Mackiewicz et McCrae, 1962 (JQ034127) was selected as the outgroup based on the results of the phylogenetic analyses by Scholz *et al.* (2021). Phylogenetic relationships were estimated using maximum likelihood (ML) and Bayesian inference (BI) methods. The best-fitting model for the analyses was estimated with jModelTest 2.1.2 (Darriba *et al.*, 2012) and was found to be the Hasegawa–Kishino–Yano model + invariant sites (HKY + I). BI analysis was conducted using MrBayes software (ver. 3.2.3) (Ronquist *et al.*, 2012). Markov chain Monte Carlo (MCMC) chains were run for 3 000 000 generations, log-likelihood scores were plotted, and only the final 75% of trees were used to produce the consensus tree. The 'burn-in' period was determined as the point when the average standard deviation of split frequency values reached <0.01. ML analysis was conducted using PhyML version 3.0 (Guindon *et al.*, 2010) run on the ATGC bioinformatics platform

Table 2. Comparative measurements of tapeworms of the long-necked *Biacetabulum*-species complex (Caryophyllidea)

Species	<i>B. isaureae</i> n. sp.	<i>B. longicollum</i> n. sp.	<i>B. overstreeti</i> n. sp.	<i>B. hypentelii</i> n. sp.
Host	<i>M. collapsum</i> <i>H. nigricans</i>	<i>M. erythrurum</i> (n = 4)	<i>M. anisurum</i> (n = 11)	<i>M. melanops</i>
Locality	South Carolina, USA	Manitoba, Canada Illinois, USA	Mississippi, USA	Tennessee, USA
Character/no. of specimens	n = 10 (mean ± s.d.)	n = 15 (mean ± s.d.)	n = 3 (mean ± s.d.)	n = 10 (mean ± s.d.)
Body length (mm)	20.6–46.3 (29.0 ± 6.22)	22.1–47.8 (32.0 ± 7.6)	–	22.0–40.6 (30.8 ± 5.6)
Maximum width	635–936 (749 ± 101)	526–948 (782 ± 137)	456–747 (629 ± 153)	580–1161 (849 ± 184)
Ratio of body width to body length	2–3% (3 ± 0.4%)	2–3% (2 ± 0.5%)	–	2–4% (3 ± 1)
Scolex length	530–721 (622 ± 71)	490–912 (754 ± 165)	562–738 (678 ± 100)	588–811 (687 ± 74)
Scolex width	524–814 (598 ± 56)	450–1022 (791 ± 186)	529–871 (700 ± 242)	659–869 (796 ± 75)
Neck length (mm)	8.6–16.7 (11.2 ± 2.8)	5.5–20.9 (12.1 ± 4.1)	3.6–7.4 (5.5 ± 2.7)	5.9–11.7 (8.9 ± 2.0)
Neck width	175–303 (234 ± 37)	152–356 (276 ± 76)	230–310 (260 ± 43)	246–471 (362 ± 64)
Ratio of neck length to body length	30–47% (38 ± 5%)	34–47% (41 ± 4%)	–	24–35% (29 ± 4%)
Testis size (length × width)	112–224 × 94–219 (n = 30)	73–191 × 68–178 (n = 45)	97–199 × 92–199 (n = 9)	81–158 × 73–150 (n = 30)
Testis number	280–555 (399 ± 89)	294–598 (449 ± 92)	–	396–533 (459 ± 42)
Distance of testes from first vitelline follicles (mm)	1.5–4.5 (2.3 ± 0.7)	1.2–5.7 (3.1 ± 1.3)	4.6	2.4–5.4 (3.3 ± 0.9)
Distance of testes from anterior extremity (mm)	10.6–21.9 (13.0 ± 2.1)	11.1–27.6 (17.5 ± 4.6)	12.7	9.4–17.9 (14.0 ± 3.3)
Distance of testes from anterior wings of ovary	216–987 (599 ± 270)	0–599 (243 ± 173)	1036–1043 (1040 ± 5)	561–1241 (906 ± 208)
Testicular area length (mm)	9.2–20.0 (13.7 ± 3.9)	6.5–16.9 (12.2 ± 3.5)	–	8.9–20.3 (14.6 ± 3.5)
Extent of testicular area in relation to body width	18–24% (21 ± 2%)	18–21% (19 ± 3%)	23–29% (25 ± 3%)	14–19% (15 ± 2%)
Cirrus-sac size (length × width)	205–261 × 192–260	140– 252 × 147– 236	139–290 × 135–274	162–232 × 151–234
Ratio of cirrus-sac width to body width	27–34% (31 ± 3%)	15–33% (26 ± 5%)	37–39% (38 ± 2%)	21–29% (25 ± 2%)
Ovary width	542–833 (577 ± 32)	338–912 (683 ± 157)	460–635 (562 ± 91)	438–997 (667 ± 164)
Length of ovarian wings	569–1380 (957 ± 225)	580–1369 (900 ± 224)	532–1150 (836 ± 257)	420–1036 (772 ± 163)
Width of ovarian wings	165–331 (217 ± 45)	128–281 (217 ± 46)	105–253 (196 ± 62)	133–267 (188 ± 38)
Ratio of length of ovarian wings to length of uterine area	39–52% (45 ± 4%)	49–61% (55 ± 4%)	35–41% (38 ± 3%)	28–50% (38 ± 6%)
Vitelline follicle size (length × width)	73–185 × 65–115 (n = 30)	51–145 × 49–114 (n = 45)	51–182 × 51–138 (n = 9)	52–121 43–88 (n = 30)
Distance of anterior-most vitelline follicles from anterior extremity (mm)	9.1–17.4 (11.4 ± 2.7)	9.4–21.9 (14.0 ± 3.5)	4.1–8.1 (6.1 ± 2.9)	6.5–12.5 (9.54 ± 2.0)
Distance of posterior-most preovarian vitelline follicles from ovary	124–458 (294 ± 125)	0–308 (132 ± 101)	212–487 (350 ± 195)	516–822 (634 ± 192)
Distance from ovary in relation to length of ovarian arms	13–48% (28 ± 12%)	0–19% (11 ± 8%)	24–42% (33 ± 13%)	40–158% (83 ± 33%)
Length of uterine area (mm)	1.5–3.2 (2.2 ± 0.5)	1.3–2.4 (1.8 ± 0.4)	1.3–2.6 (2.3 ± 0.93)	1.7–2.6 (2.1 ± 3.2)

Length of prevovarian uterine area	657–1551 (947 ± 295)	530–585 (641 ± 158)	530–1522 (1154 ± 543)	830–1291 (1055 ± 172)
Ratio of uterine area to testicular area	14–20% (16 ± 2%)	11–20% (16 ± 3%)	–	10–19% (15 ± 3%)
Size of intrauterine eggs	52–62 × 31–44 (n = 40)	46–65 × 30–41 (n = 60)	51–57 × 34–44 (n = 12)	57–64 × 36–41 (n = 40)
Distance of genital atrium from posterior extremity	1147–1933 (1542 ± 327)	839–1586 (1248 ± 266)	910–1471 (1445 ± 523)	757–1252 (1033 ± 183)
Distance of genital atrium in relation to the body length	4–6% (5 ± 0.7%)	4–5% (4 ± 0.6%)	–	3–4% (3 ± 0%)
External seminal vesicle (length × width)	174–360 × 87–127	135–292 × 46–95	188–283 × 43–96	189–440 × 56–87
Number of postovarian vitelline follicles	8–14 (10 ± 2)	12–35 (20 ± 7)	8–16 (13 ± 4)	0–13 (7 ± 4)

(<http://www.atgc-montpellier.fr/>) with a nonparametric bootstrap validation based on 100 pseudoreplicates. FigTree ver. 1.4 software (Rambaut, 2012) was used to visualise the trees. Genetic distances (uncorrected *p*-distance) were calculated in MEGA ver. 6 (Tamura *et al.*, 2013).

Morphological study

The present study is based on the evaluation of newly collected specimens of the long-necked *Biacetabulum*-species complex from four species of redhorses (*Moxostoma* spp.), spotted sucker (*M. melanops*) and northern hog sucker (*H. nigricans*) (see the list of material studied below and Table 2). Tapeworms collected by the present authors were obtained from the intestine of freshly killed fish, rinsed with saline and fixed in hot, near-boiling, 4% formaldehyde (see Appendix in Oros *et al.*, 2010). For light microscopy, specimens were stained in Mayer's carmine, dehydrated in an ascending ethanol series, cleared with eugenol, and mounted in Canada balsam. Line drawings were made using a Leica DM 5000 Blight microscope (Leica Microsystems, Wetzlar, Germany). For histological studies, the material was embedded in paraplast and 12–15 µm sections were taken and stained with Weigert hematoxylin.

For scanning electron microscopy (SEM), samples (scolecex and the posterior part of the body with gonopores) were dehydrated in ethanol and amylacetate series, infiltrated with HMDS (hexamethyldisilazane), following which the HMDS was allowed to evaporate off the specimens. Subsequently, samples were mounted on stubs, sputter-coated with gold (20–25 nm) and observed using a JEOL JSM 6510LA scanning electron microscope (JEOL Ltd., Tokyo, Japan). The terminology of microtriches follows Chervy (2009) and that of scolecex follows Mackiewicz (1994) and Oros *et al.* (2020). In morphological descriptions, measurements are in micrometres (µm) unless otherwise stated.

Scientific and common names of fish follow Froese and Pauly (2020). The newly collected material is deposited in the Harold W. Manter Laboratory, University of Nebraska, Lincoln, USA (HWML), Helminthological Collection of the Institute of Parasitology of the Biology Centre of the Czech Academy of Sciences in České Budějovice, Czech Republic (IPCAS), and the Smithsonian National Museum of Natural History, Washington, D.C., USA (USNM).

Results

Phylogeny

Both BI and ML analyses yielded trees with identical, generally strongly supported topologies (Fig. 1). Ten novel sequences of *Biacetabulum* spp. and three sequences retrieved from GenBank formed a strongly supported monophyletic group comprising the long-necked *Biacetabulum*-species complex. Within this clade, three sister species with their own unique host associations were recognised (Fig. 1).

Sequences of three novel isolates (US 272b, 272c, and 274b) and two previously published isolates (US 274c/PBI-469 and 275c/PBI-420) collected from *M. collapsum* in South Carolina, USA formed a strongly supported subclade. Sequence divergence among these isolates (intraspecific divergence) was 0–0.1% (0–1 nt).

Sequences of five novel isolates from *M. anisurum* (CAN 38c and 38d) and *M. erythrurum* (Rafinesque) (CAN 36a and 36b) in Manitoba, Canada, and from *M. erythrurum* (US 979/WV_FR19_641-2) in West Virginia, USA formed another strongly supported subclade. Sequence divergence among individual isolates in this subclade was 0–0.2% (0–2 nt).

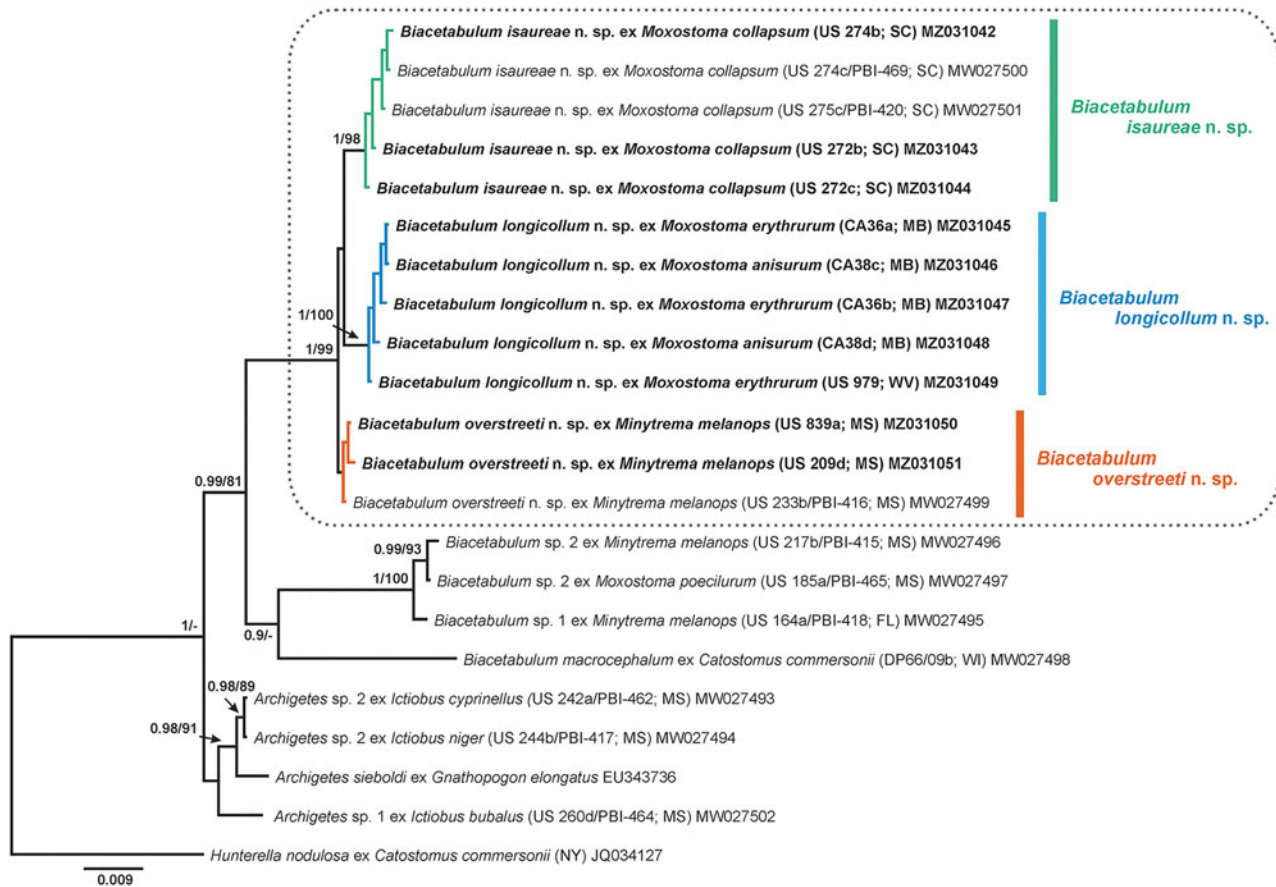


Fig. 1. Bayesian phylogram of 28S rDNA for *Biacetabulum* spp. Nodal support from BI and ML bootstrap support are indicated as BI/ML; values <0.90 (BI) and <0.70 (ML) are not shown. The scale bar indicates the expected number of substitutions per site. The newly generated sequences are highlighted in bold. The dotted rectangle indicates the clade of the long-necked *Biacetabulum*-species complex. Labels along the vertical bars reflect the species within this complex. FL, Florida; MB, Manitoba; MS, Mississippi; SC, South Carolina; WI, Wisconsin; WV, West Virginia.

Table 3. Comparative measurements of body proportion and neck length across the genus *Biacetabulum* (new species emboldened).

Species	Body length (BL) (mm)	Body width (BW) (mm)	Neck length (NL) (mm)	BW/BL %	NL/BL %
<i>B. banghami</i>	9.40	0.63	2.80	6.7	30
<i>B. biloculoides</i>	8.56	0.45	1.63	5.3	19
<i>B. carpodi</i>	5.72	0.48	0.92	8.4	16
<i>B. giganteum</i>	16.00	1.08	–	6.8	–
<i>B. hoffmani</i>	8.56	0.53	0.45	6.2	5
<i>B. infrequens</i>	22.00	0.60	0.50	2.7	2
<i>B. macrocephalum</i>	6.50	0.88	0.53	13.5	8
<i>B. meridianum</i>	15.00	0.80	–	5.3	–
<i>B. oregoni</i>	2.23	0.46	0.06	20.6	3
<i>B. isaureae</i> n. sp.	46.34	0.94	16.68	2.0	36
<i>B. longicollum</i> n. sp.	47.80	0.95	20.90	2.0	44
<i>B. overstreeti</i> n. sp.	–	0.75	7.40	–	–
<i>B. hypentelii</i> n. sp.	40.60	1.16	11.70	2.9	29

Sequences of remaining isolates, two novel (US 209d and US 839a) and one previously published (US 233b/PBI-416), collected from *M. melanops* in Mississippi, USA, formed the third subclade, albeit without strong support. These isolates represented a third species within the long-necked *Biacetabulum*-species complex. Sequence divergence among individual isolates was 0–0.1% (0–1 nt).

The interspecific divergence among the three new species ranged between 0.3 and 1.0% (4–13 nt). Phylogenetic estimates based

on sequence data also indicated notable host specificity – every species of *Biacetabulum* is restricted to one or two congeneric hosts (Fig. 1).

Morphological study

All newly collected worms of the long-necked *Biacetabulum*-species complex from different species of redhorses, spotted

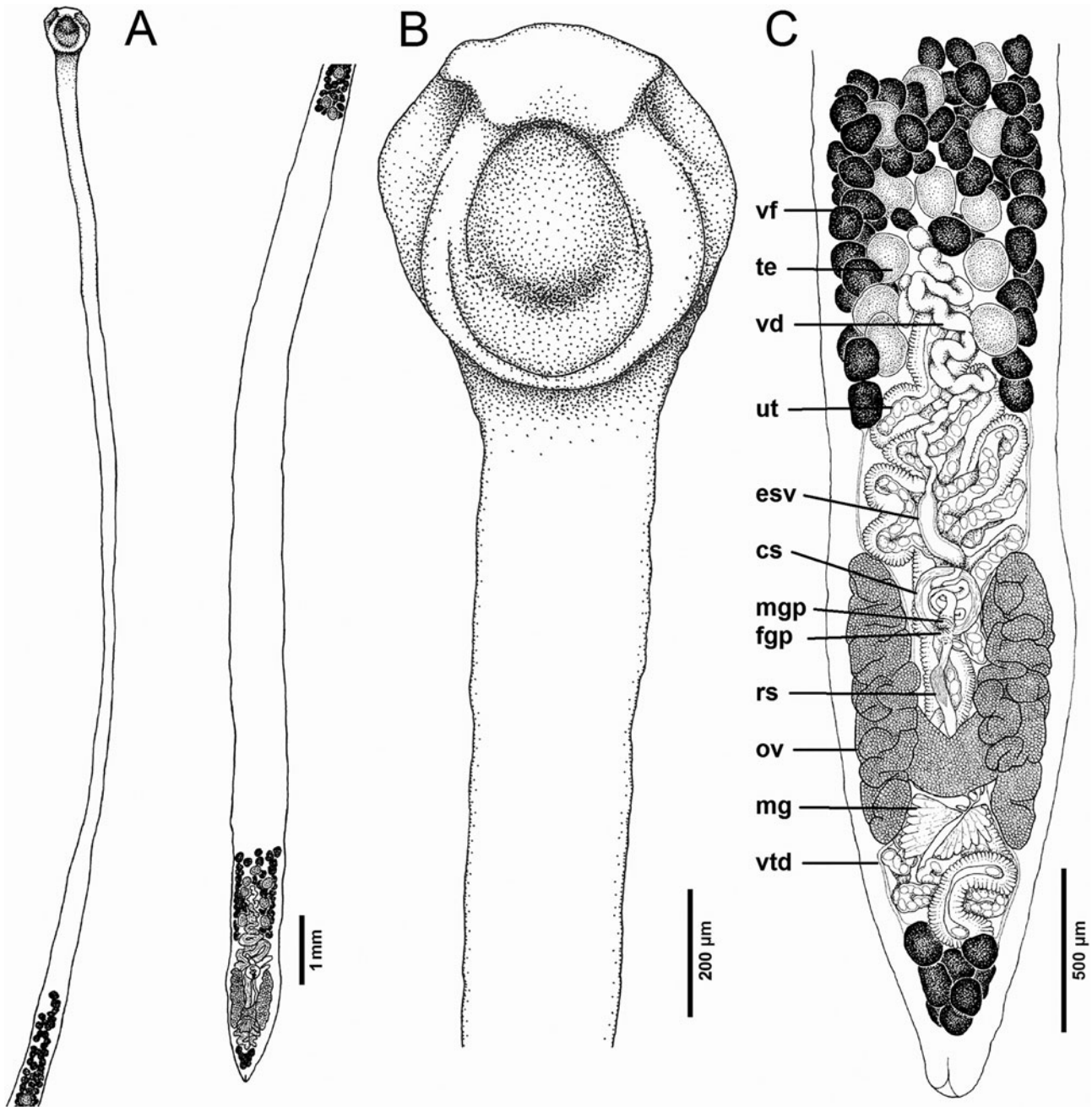


Fig. 2. Line drawings of *Biacetabulum isaureae* n. sp. from *Moxostoma collapsum*, South Carolina, USA (US 275d, IPCAS C-591/3). (A) Total view, (B) anterior part with scolex, and (C) posterior part, ventral view. cs, cirrus sac; esv, external seminal vesicle; fgp, female genital pore; mg, Mehlis' glands; mgp, male genital pore; ov, ovary; rs, seminal receptacle; te, testes; ut, uterus; vd, vas deferens; vf, vitelline follicles; vtd, vitelline duct.

sucker, and northern hog sucker are phenotypically rather uniform, being characterised by having a long body (maximum width represents only 2–3% of total body length, see Table 3) with a long, slender neck (its length represents $\geq 30\%$ of total body length, see Table 3), and a globular scolex that is much wider than the neck. The scolex bears a central, large, deep acetabulum-like loculus on the ventral and dorsal sides, shallow but distinct lateral loculi, and a distinct apical disc. All have numerous testes, a cirrus-sac that is situated between the anterior arms of ovarian wings, and a uterus that extends anteriorly well beyond the thick-walled cirrus-sac (Figs 2–8).

Examination of stained specimens (whole mounts, hologenophores or paragenophores in most cases) revealed some slight, but consistent differences between specimens of individual clades (newly described species) in the following morphological characteristics (see Table 2 for comparative measurements): (i) number

of postovarian vitelline follicles; (ii) posterior extent of preovarian vitelline follicles; (iii) relative width of the cirrus sac (ratio of the cirrus-sac width to the width of the body at its level); (iv) position of the cirrus sac in relation to the anterior wings of the ovary; (v) posterior extent of the testes; (vi) relative size of the testes; and (vii) relative extent of the uterus.

Based on molecular and morphological differences, they are considered to represent four distinct species of the long-necked *Biacetabulum*-species complex, each with its own unique host association. These new species are described below.

1. *Biacetabulum isaureae* n. sp. Figs 2–4

Material studied: 15 stained specimens (whole mounts), 2 specimens for scanning electron microscopy (SEM) and histological sections of two worms, all from notch lip redhorse, *Moxostoma*

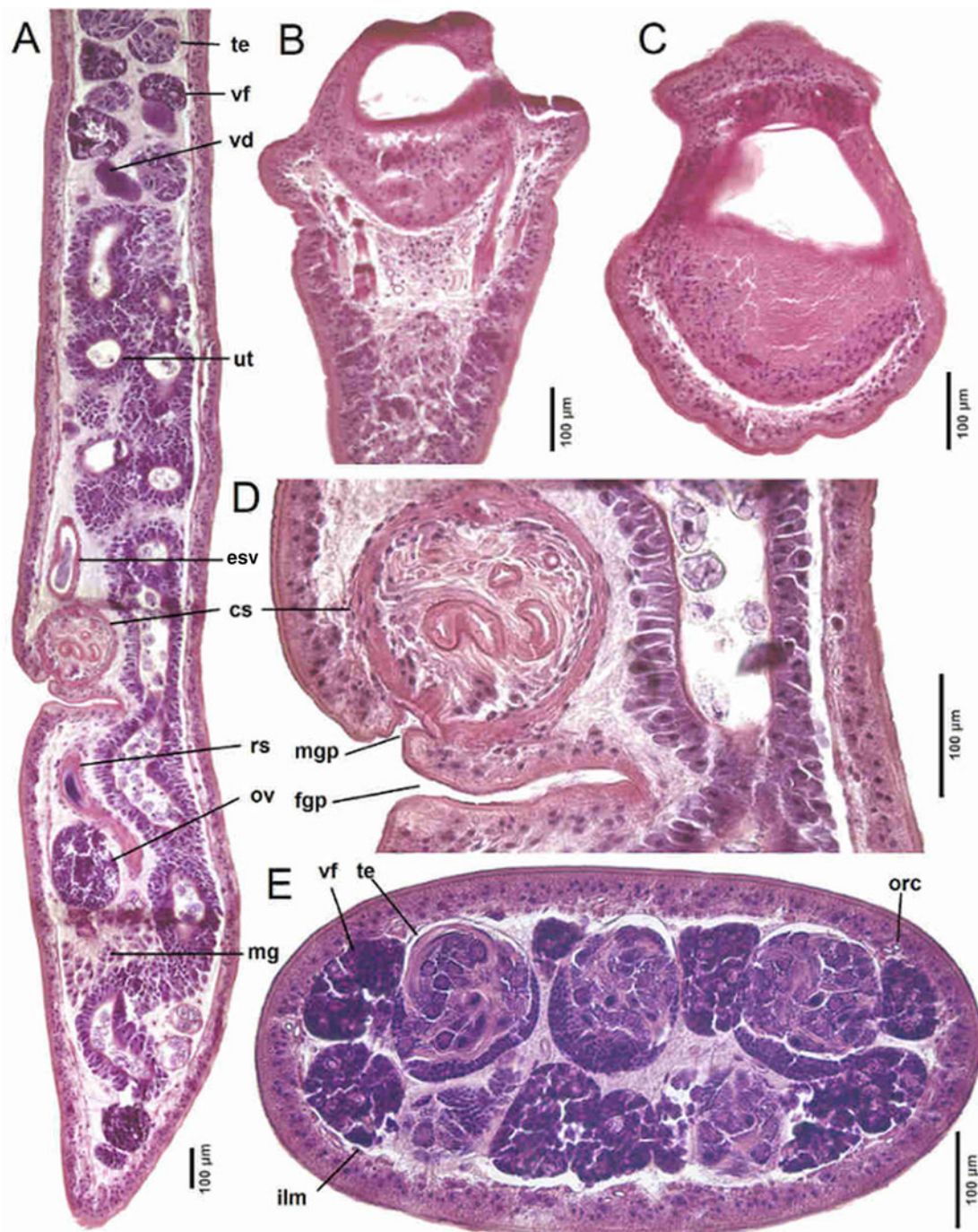


Fig. 3. Histological sections of *Biacetabulum isaureae* n. sp. from *Moxostoma collapsum* (US 275d), South Carolina, USA. (A) Sagittal section of the posterior part of body, (B, C) longitudinal sections of scolex, and (D) sagittal section of genital pores; E, cross-section of middle portion of body. cs, cirrus-sac; esv, external seminal vesicle; fgp, female genital pore; ilm, internal longitudinal muscles; mg, Mehlis' glands; mgp, male genital pore; orc, osmoregulatory canals; ov, ovary; rs, seminal receptacle; te, testes; ut, uterus; vd, vas deferens; vf, vitelline follicles.

collapsum (US 272b, 274b, 275d; IPCAS C-591/3), Congaree River at Columbia, South Carolina, USA, collected by R. Kuchta and M. Oros on 29 March 2012.

Description (based on whole mounts of 15 specimens; for measurements – see Table 2): Caryophyllidea, Capingentidae *sensu* Scholz *et al.* (2021). Body elongate and slender, with maximum width at level of anterior vas deferens or ovary, tapering towards neck region (Fig. 2A); covered with acicular fillitriches (filiform microtriches) (Fig. 4F).

Scolex spherical, wider than neck, with one pair of large acetabulum-like loculi, two pairs of shallow lateral loculi, and slightly convex apical disc (Figs 2B, 3B, C, 4A, C). Neck narrow,

very long. Internal and external longitudinal muscles well-developed. Osmoregulatory canals narrow, in cortex (Fig. 3E).

Testes medullary, subspherical to widely oval (Fig. 3E). Anterior-most testes begin posterior to anterior-most vitelline follicles. Posteriorly, testes reach anterior-most loops of uterus, slightly anterior to posterior-most preovarian vitelline follicles (Fig. 2C). Cirrus-sac subspherical, thick walled. External seminal vesicle elongate, thick walled. Male and female genital pores open to distinct genital atrium (corresponding to Fig. 5.24 of Mackiewicz, 1994; Figs 2C, 3A, D, 4B, D).

Ovary compact (non-follicular), H-shaped, with deep lobes (Fig. 2C). Vagina tubular, slightly sinuous, widened to form

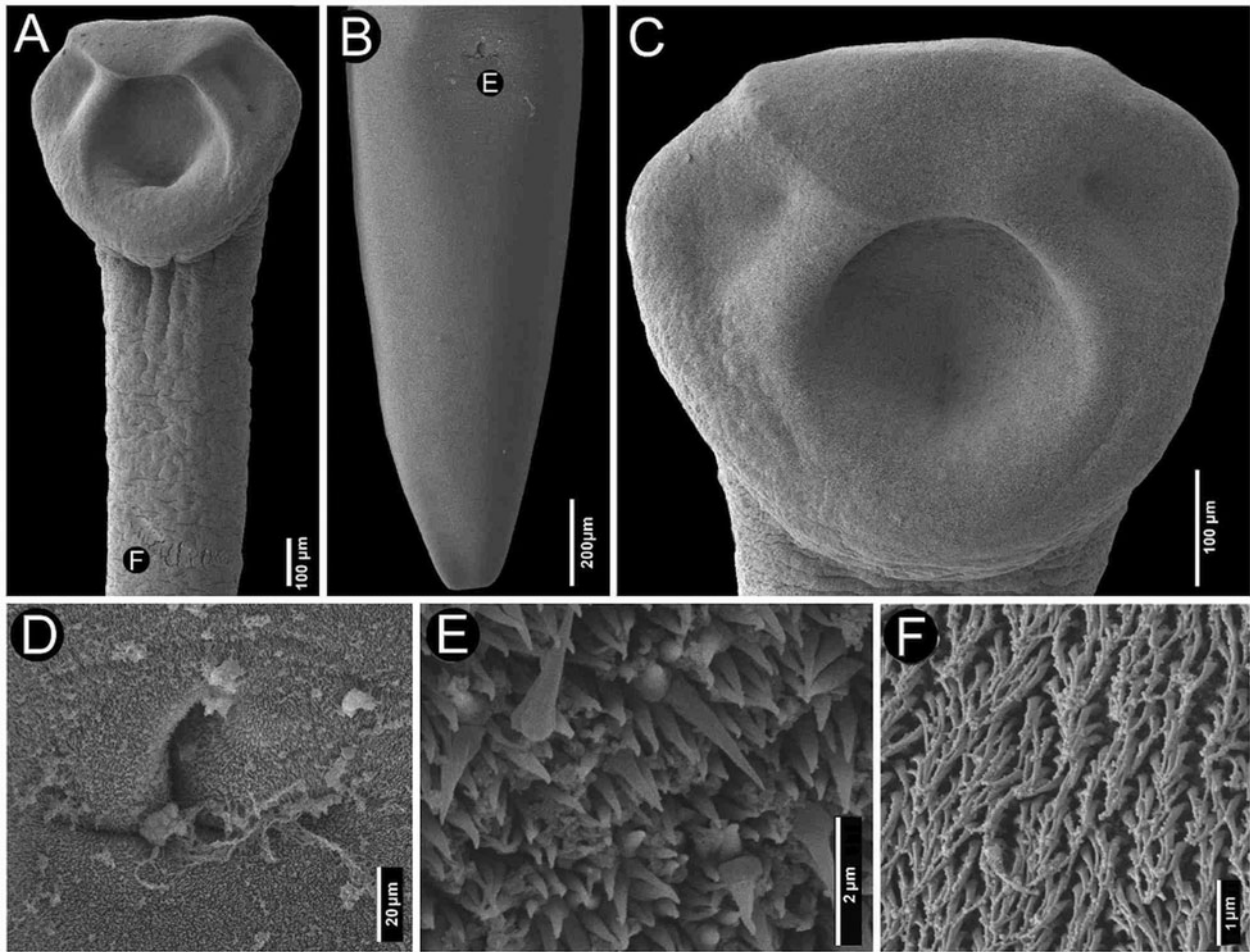


Fig. 4. Scanning electron micrographs of *Biacetabulum isaureae* n. sp. from *Moxostoma collapsum*, South Carolina, USA (US 272b, US 275d, IPCAS C-591/3). (A) Anterior part with scolex, (B) posterior part with genital pores, (C) scolex, (D) detail of common genital atrium, (E) microtriches surrounding the common genital atrium, and (F) microtriches on the body. Small letters in A and B indicate position of E and F.

elongate, narrow seminal receptacle anterior to ovarian isthmus, joins with uterus to form uterovaginal canal, opens separately from male gonopore into distinct genital atrium (Figs 3A, D, 4D). Preovarian vitelline follicles numerous, in medullary parenchyma (Fig. 3E). Preovarian vitelline follicles reach posteriorly slightly anterior to external seminal vesicle, not reaching close to ovarian wings (Fig. 2C). Postovarian vitelline follicles relatively few (Fig. 2C; Table 2).

Uterus forms several loops, extending markedly anterior to cirrus-sac (Figs 2C, 3A); uterine glands well developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without fully formed oncosphere.

Taxonomic summary

Type host: Notchlip redbhorse, *Moxostoma collapsum* (Cypriniformes: Catostomidae).

Site of infection: Usually in the anterior part of the intestine, firmly attached with the scolex, but not buried deeply into the intestinal mucosa.

Type locality: Congaree River at Columbia (33°49'48.9"N, 80°54'42.8"W), South Carolina, USA.

Distribution: USA (South Carolina).

Type material: Holotype (IPCAS C-889); two paratypes (IPCAS C-889); two paratypes (HWML 216478, 216479); two paratypes (USNM 1655788, 1655789).

Representative DNA sequences: Sequences of five individuals from *M. collapsum* (US 272b, 272c, 274b, 274c, 275c) in South Carolina were submitted to GenBank MZ031042–MZ031044.

Etymology: The species name honours Isaure de Buron, South Carolina, for her significant contribution to fish parasitology and help with sampling fishes in South Carolina in 2012.

Differential diagnosis: The new species belongs to one of three closely related, but distinct lineages of the long-necked *Biacetabulum*-species complex. Like other species of this complex described below, the new species is typified by a long body (up to 46 mm long) with a long (9–17 mm in length), slender neck, a globular scolex that is much wider than the neck region, bearing a large, deep acetabulum-like loculus on the ventral and dorsal sides, numerous testes, a cirrus sac situated between the anterior arms of ovarian lobes, and a uterus with loops extending far anterior to the thick-walled cirrus sac.

Remarks

Oros *et al.* (2020) provided an illustration (Fig. 3C) and SEM micrograph (Fig. 6D) of the scolex of *B. isaureae* n. sp. from *M. collapsum*, but the tapeworm was misidentified as *B. infrequens* because of a resemblance of their scoleces. However, a closer examination of this tapeworm revealed that it is *B. isaureae*. In addition, the worm in Oros *et al.* (2020) was collected from *M. collapsum* and not the spotted sucker, *M. melanops*, as was reported.

2. *Biacetabulum longicollum* n. sp. Figs 5, 6

Material studied: 12 stained specimens (whole mounts) from *Moxostoma anisurum*, Assiniboine River, Manitoba, Canada, collected by P.A. Nelson on 22 May 1996; 23 specimens from

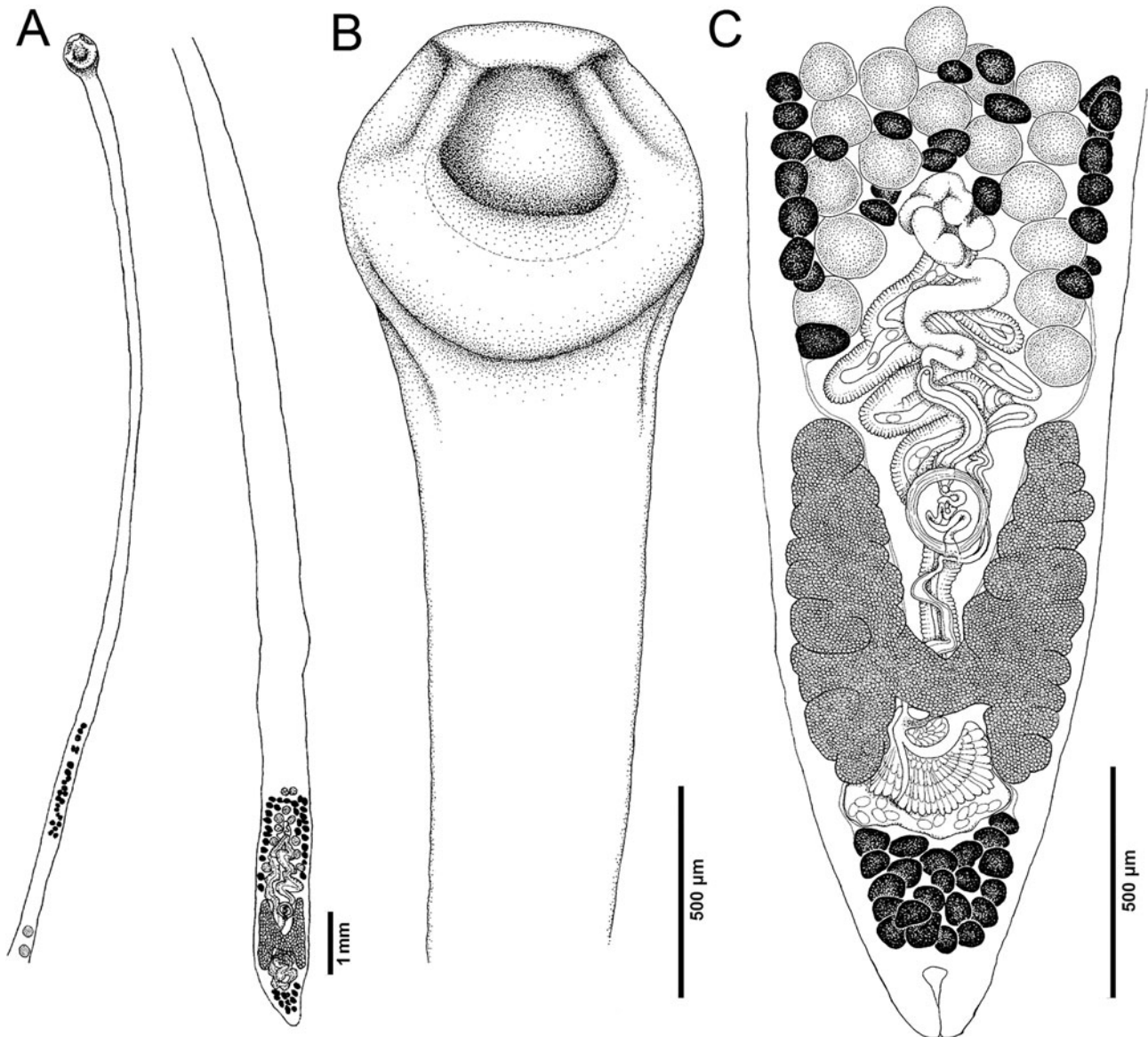


Fig. 5. Line drawings of *Biacetabulum longicollum* n. sp. from *Moxostoma anisurum*, Red River, St. Vital Park, Manitoba, Canada (CA 38), and Assiniboine River, Manitoba, Canada (SR-I-96M). (A) Total view, (B) anterior part with scolex, and (C) posterior part, ventral view.

M. anisurum [host field code CA (= Canada) 38, 39, 52], Red River, St. Vital Park, Manitoba, Canada, A. Choudhury and M. Oros, 26 and 27 June 2013; 7 specimens from *Moxostoma erythrurum* (CA 36), Red River, St. Vital Park, Manitoba, Canada, A. Choudhury and M. Oros, 26 June 2013; 1 specimen from *M. erythrurum* [FR19_641 (= Florian Reyda's collection)], Kanawha River, Montgomery, West Virginia, F. Reyda, 9 August 2019.

Description (based on whole mounts of 13 specimens from *M. anisurum* in Canada; for measurements – see Table 2): Caryophyllidea, Capingentidae *sensu* Scholz *et al.* (2021). Body elongate, slender, with maximum width at level of anterior vas deferens, or ovary, tapering towards neck region (Fig. 5A), covered with acicular fillitriches (filiform microtriches) (Fig. 6F).

Scolex spherical, wider than neck, with pair of large, central acetabulum-like loculi, two pairs of shallow lateral loculi, and slightly convex apical disc (Figs 5B, 6A, C). Neck narrow, very long. Internal and external longitudinal muscles well developed. Osmoregulatory canals narrow, in cortex.

Testes medullary, subspherical to widely oval. Anterior-most testes begin posterior to anterior-most vitelline follicles. Posteriorly, testes reach halfway down anterior uterine loops,

relatively close to ovary, slightly short of posterior-most preovarian vitelline follicles. Cirrus-sac subspherical, thick walled. External seminal vesicle elongate, thick walled. Male and female genital pores open to distinct genital atrium (corresponding to figure 5.24 of Mackiewicz, 1994; Figs 5C, 6D), area around gonopores covered with gladiate spinitriches (Fig. 6E).

Ovary compact (non-follicular), H-shaped, with deep lobes (Fig. 5C). Vagina tubular, slightly sinuous, widened to form elongate, narrow seminal receptacle anterior to ovarian isthmus, joins uterus to form uterovaginal canal, opening separately from male gonopore in distinct genital atrium. Preovarian vitelline follicles numerous, in medullary parenchyma. Preovarian vitelline follicles extend beyond testes, terminating slightly anterior to external seminal vesicle, relatively close to ovarian wings (Fig. 5C). Postovarian vitelline follicles present, numerous (Fig. 5C; Table 2).

Uterus forms several loops, extending markedly anterior to cirrus-sac; preovarian uterine loops occupy roughly triangular space (Fig. 5C); uterine glands well developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without fully formed oncosphere.

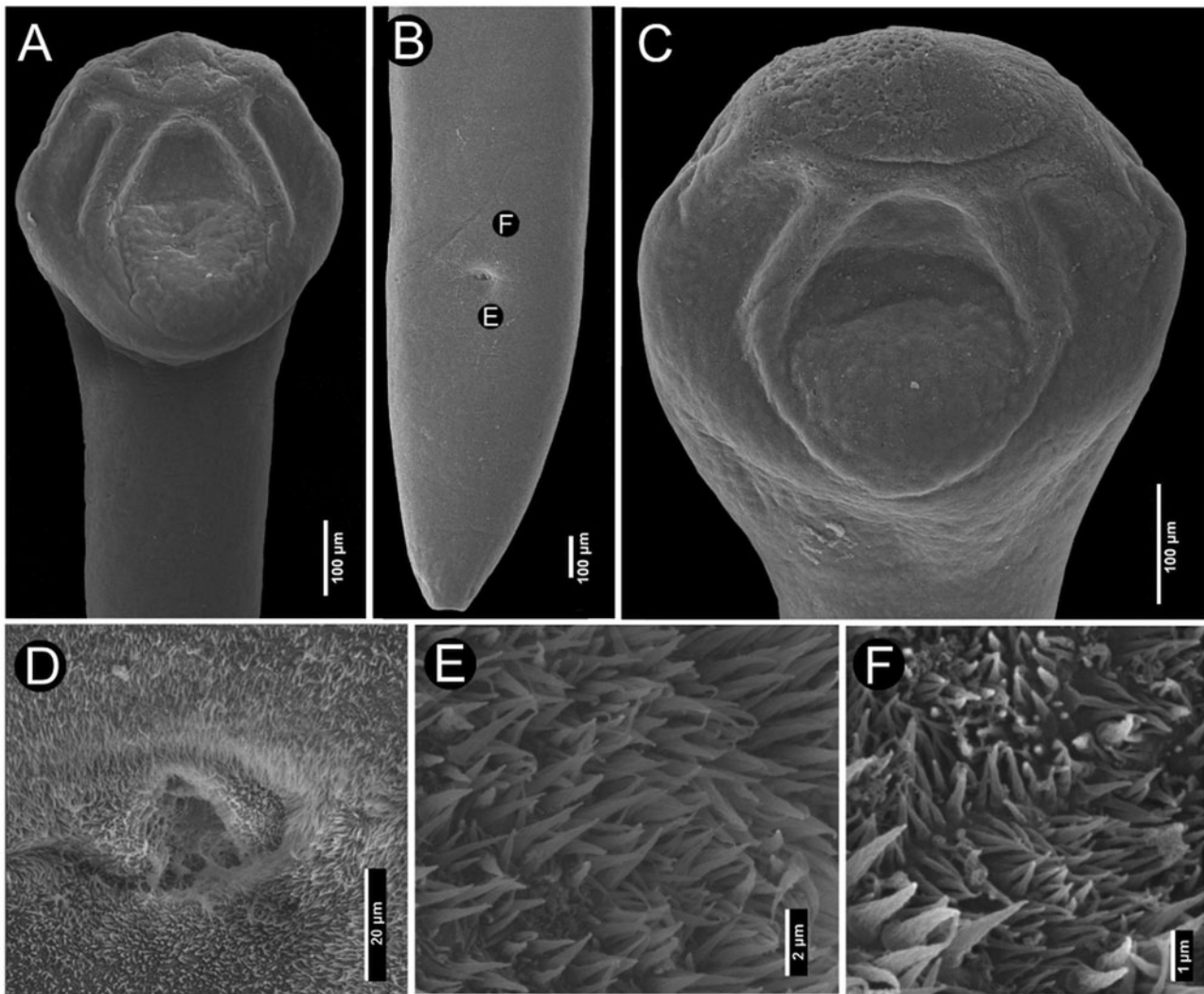


Fig. 6. Scanning electron micrographs of *Biacetabulum longicollum* n. sp. from *Moxostoma anisurum*, Red River, Manitoba, Canada (CA 38). (A) Anterior part with scolex, (B) posterior part with genital pores, (C) scolex, (D) detail of common genital atrium, and (E) microtriches surrounding the common genital atrium. Small letters in B indicate position of E and F.

Taxonomic summary

Type host: Silver redhorse, *Moxostoma anisurum* (Cypriniformes: Catostomidae).

Additional definitive host (verified by molecular data): Golden redhorse, *M. erythrurum* (Cypriniformes: Catostomidae).

Site of infection: Usually in the anterior part of the intestine, firmly attached with the scolex, but not buried deeply into the intestinal mucosa.

Type locality: Red River, St. Vital Park (49°49'49.9"N, 97°8'53.8"W), Manitoba, Canada.

Distribution: Canada (Manitoba), USA (West Virginia).

Type material: Holotype (IPCAS C-890/1); two paratypes (IPCAS C-890/1); two paratypes (HWML 216480); two paratypes (USNM 1655790).

Representative DNA sequences: Sequences of two individuals from *M. anisurum* (CA 36a, 36b) in Manitoba, Canada and three individuals from *M. erythrurum* (CA 38c, 38d, FR19_641) in Manitoba, Canada and West Virginia, USA were submitted to GenBank MZ031045–MZ031049.

Etymology: The species is named after the typical, very long and narrow neck – longi = long, colli = neck.

Differential diagnosis: The new species differs from *B. isaureae* n. sp. by (i) the extent of the ovarian wings in relation to the extent of the area occupied by the uterus, 49–61% in

B. longicollum n. sp. vs 39–52% in *B. isaureae*; (ii) the extent of preovarian vitelline follicles, which reach relatively close to the ovarian wings in *B. longicollum* vs slightly anterior to the external seminal vesicle in *B. isaureae*; (iii) position of the cirrus sac, which is situated more posteriorly between the anterior ovarian wings in *B. longicollum* compared to *B. isaureae*; and (iv) more numerous postovarian follicles in *B. longicollum* (12–35) compared to *B. isaureae* (8–14 follicles).

Remarks

Molecular data provide evidence for tapeworms from *M. anisurum* and *M. erythrurum* in Canada and West Virginia to belong to a separate lineage than those from *M. collapsum* in South Carolina, differing in 8–13 nucleotides (interspecific divergence 0.6–1.0%). Morphological differences between *B. longicollum* n. sp. and *B. isaureae* n. sp. are subtle, but consistent. Taken together, the molecular and morphological data indicate that they represent closely related, but distinct species. *Biacetabulum longicollum* and *B. isaureae* both occur in congeneric hosts (*Moxostoma* spp.). Future surveys should provide more data on the actual host spectrum and distribution of this newly described species, which is currently known from Manitoba (Canada) and West Virginia (USA).

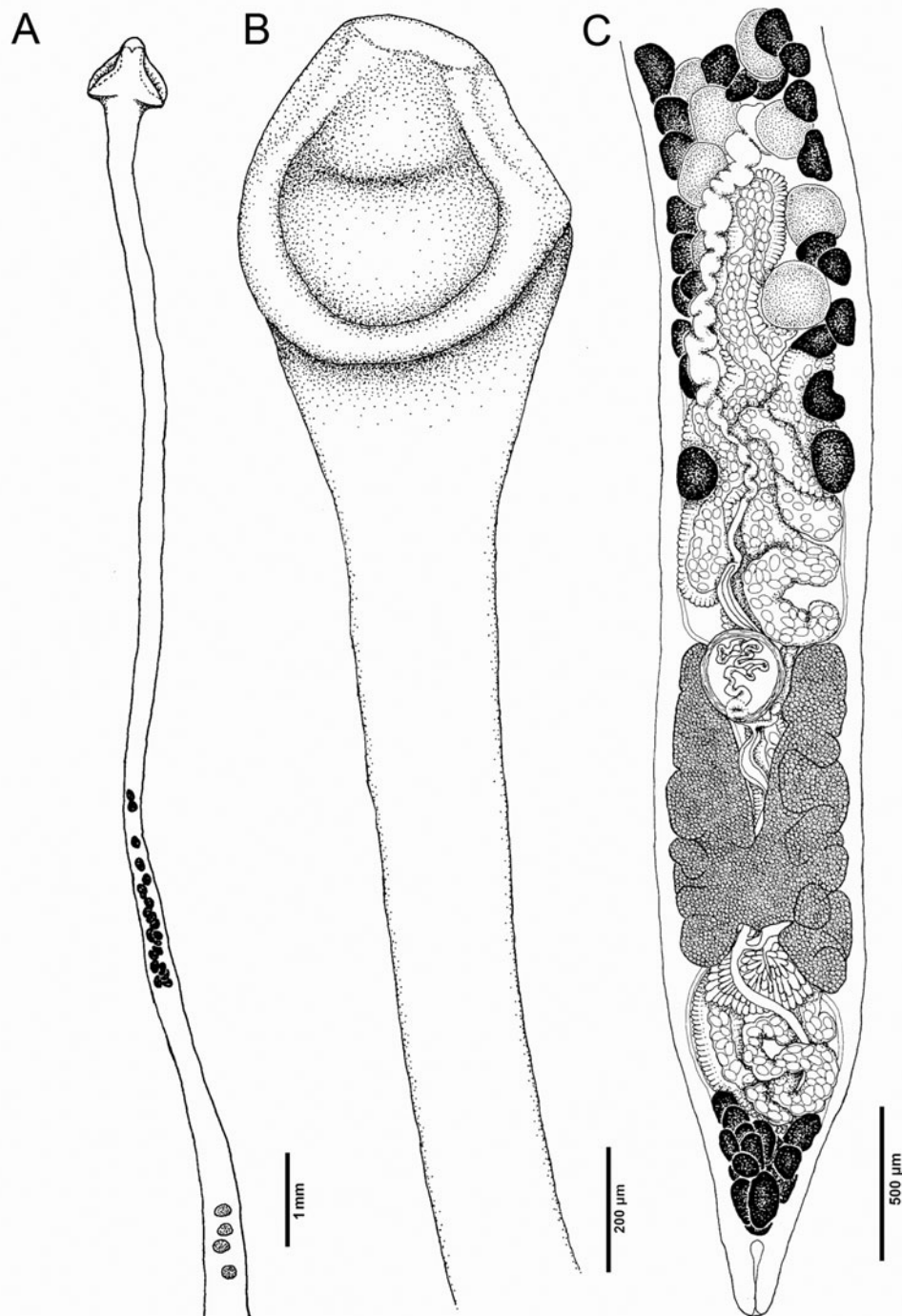


Fig. 7. Line drawings of *Biacetabulum overstreeti* n. sp. from *Minytrema melanops*, Mississippi, USA (US 209d, US 233b, US 839a). (A) Total view, (B) anterior part with scolex, and (C) posterior part, ventral view.

In addition to *B. longicollum* n. sp., another species, *B. infrequens* Hunter, 1927, was described from *M. anisurum* by Hunter (1927). Even though the original description of *B. infrequens* was brief and no illustration of the whole worm was provided by Hunter (1927), it is possible to differentiate this species from *B. longicollum* n. sp. by the following characteristics (compare Figs 5, 6 in the present paper with figs. 2, 3, 13–15 in Hunter, 1927 and figs. 15, 16 in Calentine, 1965): (i) total body length (16–22 mm in *B. infrequens* vs up to 48 mm in *B. longicollum* n. sp.); (ii) length of the neck (short, only 0.5 mm in length in *B. infrequens* vs very long, 5.5–20.9 mm in the new species); (iii) position of the cirrus sac (anterior to the ovary in the former species vs between the anterior ovarian arms in *B. longicollum*

n. sp.); and (iv) scolex morphology (see Hunter, 1927 and Calentine, 1965).

SEM examination of *B. longicollum* has revealed the presence of two different types of microtriches around the genital pores: numerous, dense acicular filitriches are intermingled with relatively few gladiate spinitriches (Fig. 6E, F). As far as the present authors are aware, detailed information about the surface ultrastructure (microtrich morphology) of caryophyllideans around the gonopore is not available, thus further research is needed, including SEM and detailed transmission electron microscopy observations, to clarify the different types of microtriches. Overall, the surface of caryophyllidean tapeworms has been reported to be uniformly covered by the acicular or capilliform

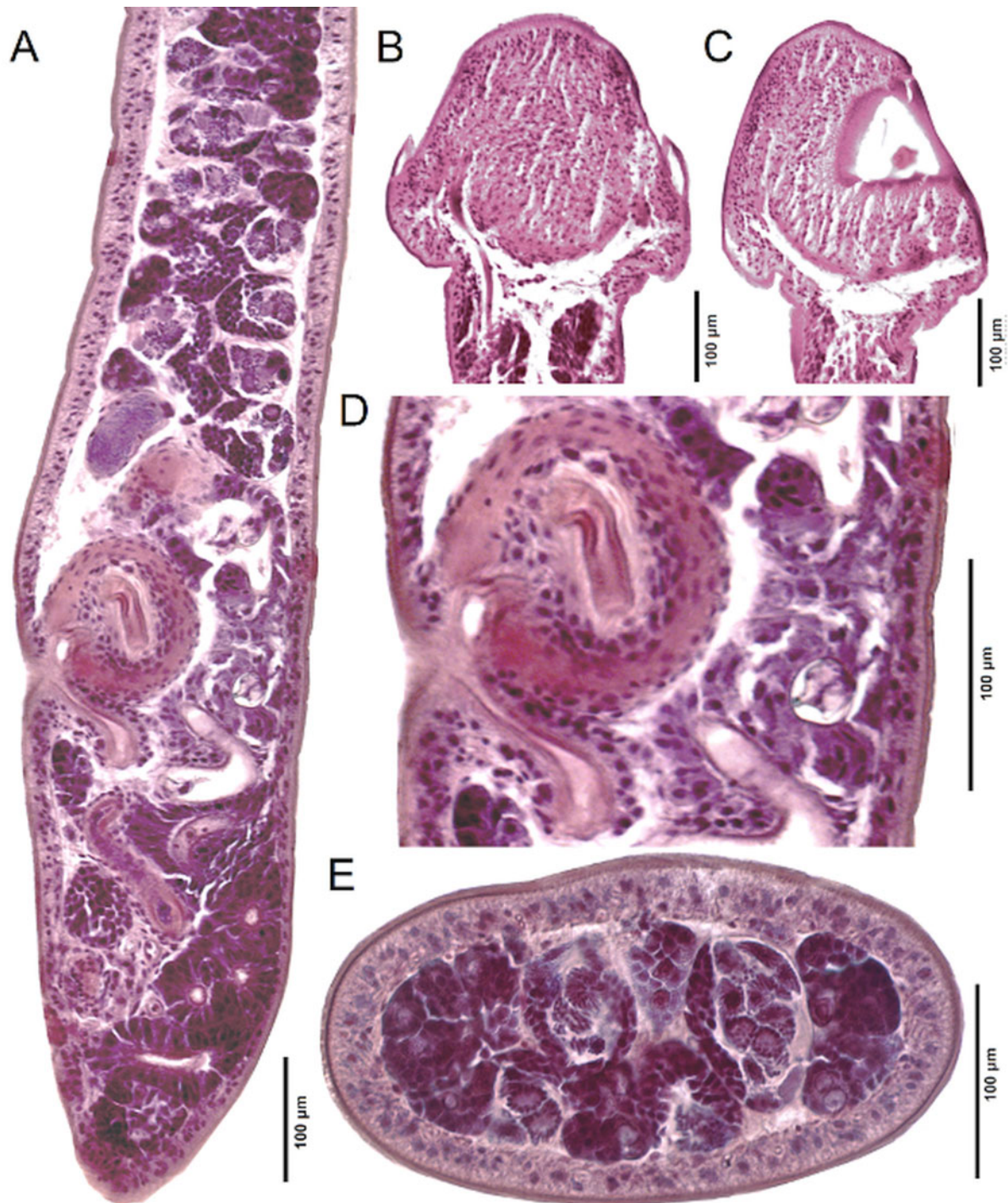


Fig. 8. Histological sections of *Biacetabulum overstreei* n. sp. from *Minytrema melanops*, Mississippi, USA (US 209d, US 224a). (A) Sagittal section of the posterior part of body, (B, C) longitudinal section of scolex, (D) sagittal section of genital pores, and (E) cross-section of the middle portion of body.

filitrices (see, e.g., Ash *et al.*, 2011; Scholz *et al.*, 2015; Oros *et al.*, 2016; Barčák *et al.*, 2017).

3. *Biacetabulum overstreei* n. sp. Figs 7–9

Material studied: One specimen (hologenophore, comprising the anterior and posterior part of the specimen) including its cross-sections from *Minytrema melanops* (US 209d), Big Lake near Benndale, Pascagoula River, Mississippi, collected by R. Kuchta and M. Oros on 20 March 2012; one specimen (hologenophore – PBI 416) from *M. melanops* (US 233b), Benndale, Pascagoula River, Mississippi, R. Kuchta and M. Oros, 23 March 2012; one

specimen (hologenophore) from *M. melanops* (US 839a), Poticaw Landing & Moon Lake, Pascagoula River, Mississippi, T. Scholz, R. Kuchta and M. Oros, 20 June 2019; longitudinal sections of a specimen from *M. melanops* (US 224a), Pearl River, Mississippi (30°52'20.2"N, 88°46'21.8"W), R. Kuchta and M. Oros, 22 March 2012.

Description (based on whole mounts of three specimens from *M. melanops* in Mississippi, USA; for measurements, see Table 2): Caryophyllidea, Capingentidae *sensu* Scholz *et al.* (2021). Body elongate and slender, with maximum width at level of anterior vas deferens or ovary, tapering continuously towards neck region, covered with acicular filitrices (filiform microtriches) (Fig. 9C).

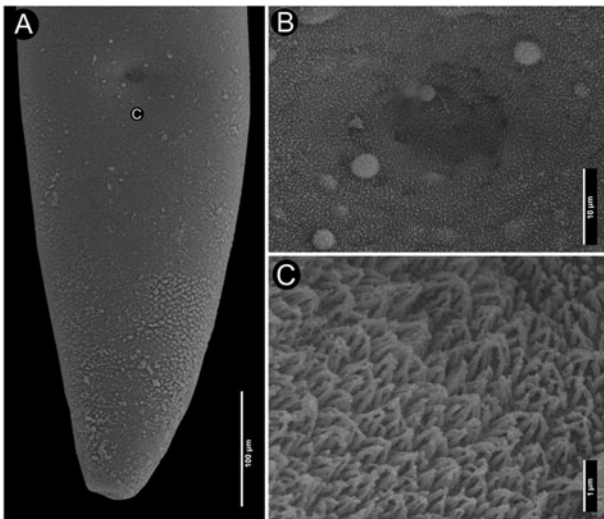


Fig. 9. Scanning electron micrographs of *Biacetabulum overstreeti* n. sp. from *Minytrema melanops*, Mississippi, USA (US 224a). (A) Posterior part with genital pores, (B) detail of common genital atrium, and (C) microtriches surrounding the common genital atrium.

Scolex spherical, wider than neck, with pair of central, large acetabulum-like loculi, two pairs of shallow lateral loculi, and slightly convex apical disc (Figs 7A, B, 8B, C). Neck narrow, very long. Internal and external longitudinal muscles well developed. Osmoregulatory canals narrow, in cortex (Fig. 8E).

Testes medullary, subspherical to widely oval (Fig. 8E). Anterior-most testes begin posterior to anterior-most vitelline follicles. Posteriorly, testes reach anterior-most loops of uterus, slightly anterior to posterior-most preovarian vitelline follicles (Fig. 7C). Cirrus-sac subspherical, thick-walled. External seminal vesicle elongate, thick-walled. Male and female genital pores open into distinct genital atrium (corresponding to figure 5.24 of Mackiewicz, 1994; Figs 7C, 8A, D, 9A, B).

Ovary compact (non-follicular), H-shaped, with deep lobes (Fig. 7C). Vagina tubular, slightly sinuous, widened to form elongate, narrow seminal receptacle anterior to ovarian isthmus, joins with uterus to form uterovaginal canal opening posterior to male gonopore into distinct genital atrium (Figs 8A, D, 9A, B). Preovarian vitelline follicles numerous, in medullary parenchyma (Fig. 8E). Preovarian vitelline follicles reach posteriorly anterior to external seminal vesicle, not extending to ovarian wings (Fig. 7C). Postovarian vitelline follicles few (Fig. 7C; Table 2).

Uterus forms several loops extending markedly anterior to cirrus-sac (Figs 7C, 8A); uterine glands well developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without a fully formed oncosphere.

Taxonomic summary

Type and only host: Spotted sucker, *Minytrema melanops* (Cypriniformes: Catostomidae).

Site of infection: Usually in the anterior part of the intestine; firmly attached with the scolex, but not buried deeply into the intestinal mucosa.

Type locality: Pascagoula River at Benndale (30°52'20.2"N, 88°46'21.8"W), George County, Mississippi, USA, R. Kuchta and M. Oros, 20 March 2012, USA.

Distribution: USA (Mississippi).

Type material: Holotype (IPCAS C-891); one paratype (longitudinal sections) (IPCAS C-891); two paratypes (whole mount – hologenophore and longitudinal sections) (USNM 1655791, 1655792).

Representative DNA sequences: Sequences of three individuals from *M. melanops* (US 209d, 233b and 839a) in Mississippi were submitted to GenBank MZ031050, MZ031051.

Etymology: The species name is dedicated to Robin M. Overstreet from the Gulf Coast Research Laboratory, Ocean Springs, Mississippi, for his extraordinary contribution to fish parasitology and for supporting the present authors in their sampling trips to southern Mississippi in 2012 and 2019.

Differential diagnosis: The new species corresponds in its morphology to other species of the long-necked *Biacetabulum*-species complex characterised above. It differs from *B. isaureae* n. sp. and *B. longicollum* n. sp. mainly by the size and position of the cirrus-sac. The width of the cirrus-sac represents more than one-third (37–39%) of the body width at the level of the cirrus-sac in *B. overstreeti* (*B. isaureae* 27–34%; *B. longicollum* 24–28%). The cirrus-sac is situated slightly before the anterior end of the ovarian wings in *B. overstreeti* vs being situated more posterior, i.e., between the ovarian arms in *B. isaureae* and *B. longicollum*.

In addition, *B. overstreeti* differs in the length of the ovarian wings in relation to the length of the uterus area, which is 35–41% in *B. overstreeti* vs 39–52% in *B. isaureae* and 49–61% in *B. longicollum*. The new species can also be differentiated from *B. longicollum* by a lower number of postovarian vitelline follicles (usually 8–16 follicles) compared to 12–35 follicles in the latter species (see also Table 2 for comparative measurements and the key for identifying species of the 'long-necked' species complex provided below).

Molecular data provide additional evidence that tapeworms from *M. melanops* collected in Mississippi, USA belong to a separate lineage and differ from *B. isaureae* in 4–8 nucleotides (inter-specific divergence 0.3–0.6%) and from *B. longicollum* in 6–9 nucleotides (0.5–0.7%).

Remarks

Only three hologenophores of *B. overstreeti* n. sp. were available for morphological descriptions of this species. Therefore, the total length of the body could not be measured. Despite this limitation, we are fully convinced that other morphological and biometrical characteristics are available to sufficiently characterize the new species, which is well characterized also genetically (Fig. 1).

Another species of *Biacetabulum*, *B. banghami* Mackiewicz, 1968, was described from *M. melanops* in Alabama, but this species has also been reported from *Moxostoma erythrurum* and *H. etowanum* (Jordan) in Oklahoma and Kentucky (Mackiewicz, 1968). This species differs from *B. overstreeti* n. sp. most conspicuously by scolex morphology (the scolex of *B. banghami* bears very shallow loculi and two pairs of auricular projections on its anterior edge – see Mackiewicz, 1968).

4. *Biacetabulum hypentelii* n. sp. Figs 10, 11

Material studied: 15 stained specimens (whole mounts) and 2 specimens for SEM examination from *Hypentelium nigricans* (851, 852), Hiwassee River downstream Apalachia Dam, Tennessee, USA, collected by Price, Sewell, Entier, Bouchard and J.S. Mackiewicz on 5 May 1968.

Description (based on whole mounts of 15 specimens from *H. nigricans*, Tennessee, USA; for measurements – see Table 2): Caryophyllidea, Capingentidae *sensu* Scholz *et al.* (2021). Body elongate, slender, with maximum width at level of anterior vas deferens or ovary, tapering towards neck region (Fig. 10A). Body covered with acicular fillitriches (filiform microtriches) (Fig. 11F).

Scolex spherical, wider than neck, with pair of large, central acetabulum-like loculi, two pairs of shallow lateral loculi, and

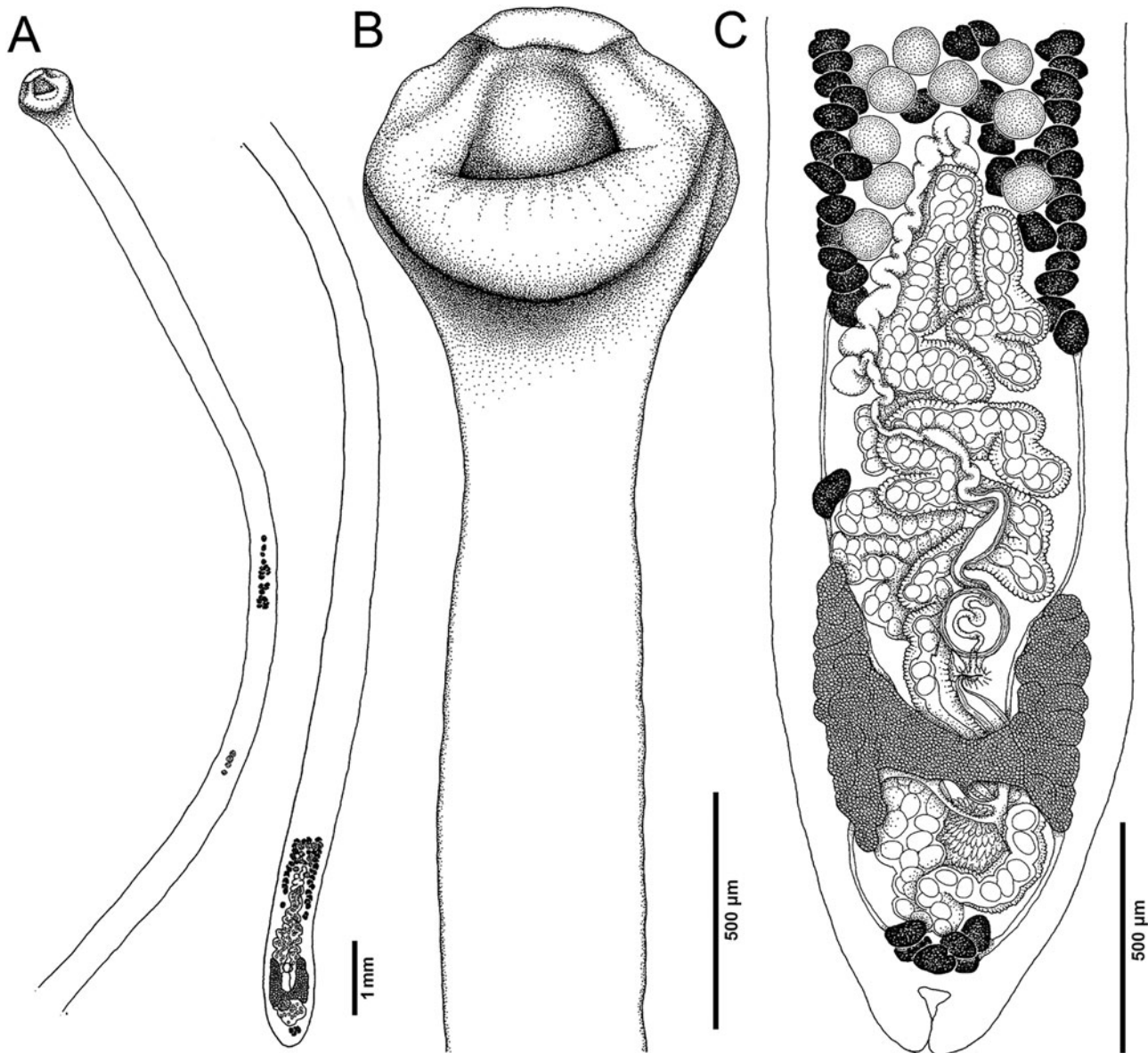


Fig. 10. Line drawings of *Biacetabulum hypentelii* n. sp. from *Hypentelium nigricans*, Tennessee, USA (851). (A) Total view, (B) anterior part with scolex, and (C) posterior part, ventral view.

slightly convex apical disc (Figs 10B, 11A, C). Neck narrow, very long. Internal and external longitudinal muscles well-developed.

Testes medullary, subspherical to widely oval. Anterior-most testes begin posterior to anterior-most vitelline follicles. Posteriorly, testes reach halfway down anterior uterine loops, relatively close to ovary, slightly short of posterior-most preovarian vitelline follicles. Cirrus-sac subspherical, thick walled. External seminal vesicle elongate, thick walled. Male and female genital pores open to distinct genital atrium (corresponding to figure 5.24 of Mackiewicz, 1994), (Fig. 11D), area around gonopores covered with gladiate spinitrices (Fig. 11E).

Ovary compact (non-follicular), H-shaped, with deep lobes (Fig. 10C). Vagina tubular, slightly sinuous, widened to form elongate, narrow seminal receptacle anterior to ovarian isthmus, joins uterus to form uterovaginal canal, opening separately from male gonopore in distinct genital atrium. Preovarian vitelline follicles numerous, in medullary parenchyma. Preovarian vitelline follicles extend beyond testes, terminating slightly anterior to external seminal vesicle, relatively far to ovarian wings (Fig. 10C). Postovarian vitelline follicles in low number, sometimes absent (Fig. 10C; Table 2).

Uterus forms several loops, extending markedly anterior to cirrus-sac; preovarian uterine loops occupy roughly triangular space (Fig. 10C); uterine glands well-developed, absent only in most distal and proximal parts of uterus. Eggs operculate, without a fully formed oncosphere.

Taxonomic summary

Type host: Northern hog sucker, *Hypentelium nigricans* (Cypriniformes: Catostomidae).

Site of infection: Intestinal lumen (more precise data not available).

Type locality: Hiwassee River downstream Apalachia Dam, Tennessee, USA (35°10'07.0"N 84°17'54.9"W).

Distribution: USA (Tennessee).

Type material: Holotype (IPCAS C-892); two paratypes (IPCAS C-892); two paratypes (HWML 216481); two paratypes (USNM 1655793, 1655794).

Etymology: The species is named after the type host.

Differential diagnosis: The new species differs from the other three new species by (i) less numerous postovarian follicles, even absent in *B. hypentelii* (0–13 follicles) vs 8–14 in *B.*

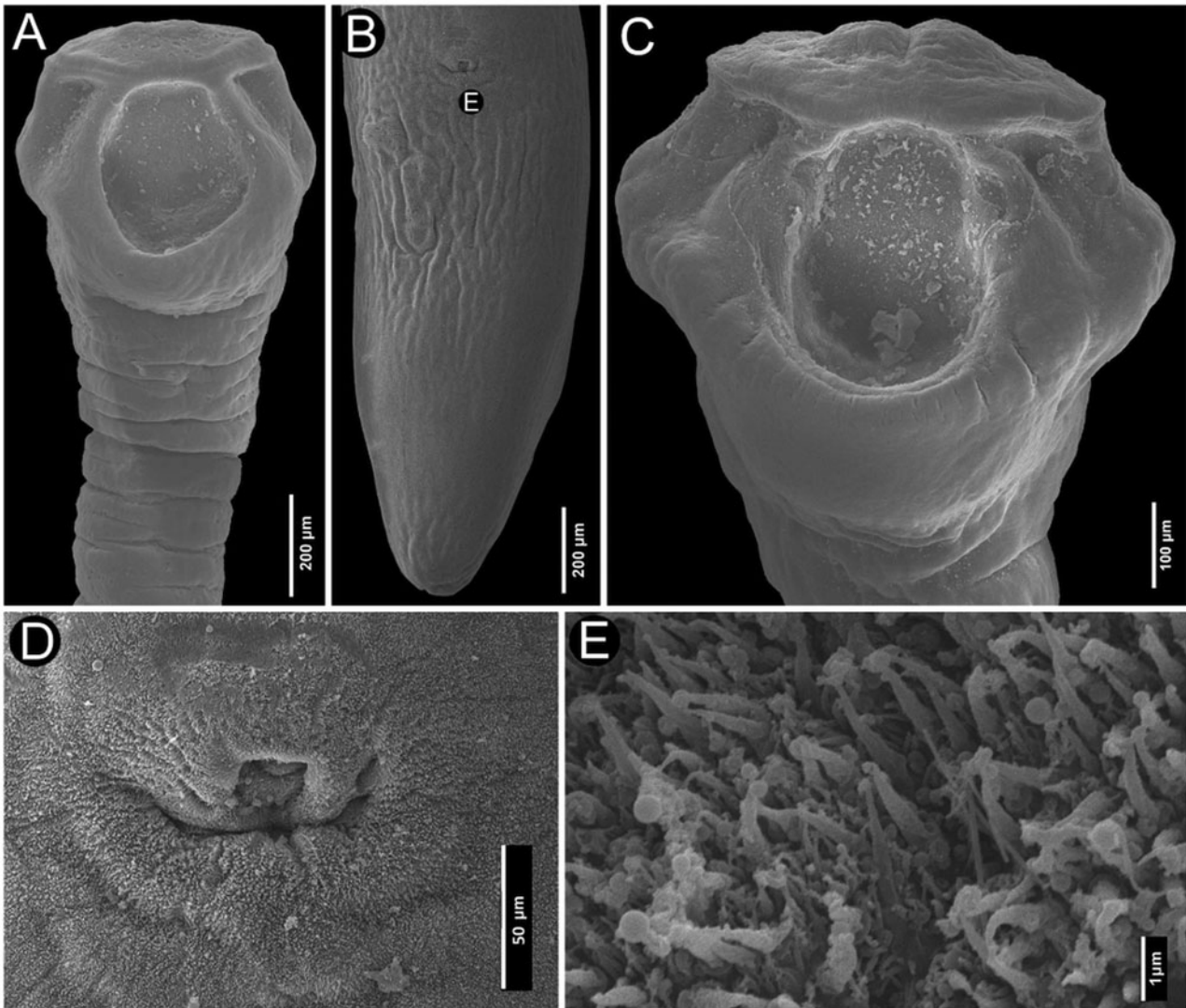


Fig. 11. Scanning electron micrographs of *Biacetabulum hypentelii* n. sp. from *Hypentelium nigricans*, Tennessee, USA (851). (A) Anterior part with scolex, (B) posterior part with genital pores, (C) scolex, (D) detail of common genital atrium, and (E) microtriches surrounding the common genital atrium.

isaureae, 12–35 in *B. longicollum* and 8–16 in *B. overstreeti*; (ii) extent of preovarian vitelline follicles, which reach relatively far from the ovarian wings in *B. hypentelii* vs slightly anterior to the external seminal vesicle in *B. isaureae* and *B. overstreeti*, and relatively close to the ovarian wings in *B. longicollum*; (iii) relative size of the cirrus in relation to the body with in *B. hypentelii* 21–29% vs 27–34% in *B. isaureae*, 15–33% in *B. longicollum*, and 37–39% in *B. overstreeti*; (iv) length of neck in relation to total body length in *B. hypentelii* 24–35% vs 30–47% in *B. isaureae* and 34–47% in *B. longicollum*; (v) the extent of the ovarian wings in relation to the extent of the area occupied by the uterus, 28–50% in *B. hypentelii* vs 39–52% in *B. isaureae* 49–61% in *B. longicollum* and 35–41% in *B. overstreeti*; (vi) position of the genital atrium from posterior margin in relation to total body length, 3–4% in *B. hypentelii* vs 4–5% in *B. longicollum* and 4–6% in *B. isaureae*; and (vii) size of testes in relation to body width in *B. hypentelii* 14–19% vs 18–24% *B. isaureae*, 18–21% in *B. longicollum* and 23–29% in *B. overstreeti*.

Remarks

Mackiewicz (1972) provided a microphotograph (see his fig. 6) of a long-necked tapeworm identified as *B. infrequens* that he found

in two northern hogsuckers, *H. nigricans*, from the Hiwassee River in Tennessee in May 1968. These specimens were kindly provided by J.S. Mackiewicz and examined; they resemble those of the long-necked *Biacetabulum*-species complex described in this study. Although no ethanol-preserved material was available for the present molecular analyses, these specimens differ from the three recently described species (see above), and represent a new species.

A key to the identification of species of the long-necked *Biacetabulum*-species complex

Because of close morphological similarity in the sibling species of the long-necked *Biacetabulum*-species complex characterised above, a key to their identification is provided. In some cases, measurements of individual species may overlap; therefore, it is necessary to combine several characteristics.

- 1a. Postovarian follicles numerous, up to 35 (mean > 10) 2.
- 1b. Postovarian follicles few, <13 (mean 7); preovarian vitelline follicles and testes reach the level of the external seminal vesicle, i.e., relatively far from the anterior margin of the ovary (Fig. 5C); cirrus-sac situated more posteriorly between the

- ovarian wings (Fig. 5C); parasite of *Hypentelium nigricans* *B. hypentelii* n. sp.
- 2a. Preovarian vitelline follicles and especially testes do not reach the level of the external seminal vesicle, i.e., they are at distance from the anterior margin of the ovary (Figs 2C, 7C); cirrus-sac situated more anterior between the ovarian wings (Figs 2C, 7C)..... 3
- 2b. Preovarian vitelline follicles and testes reach more posteriorly, up to the level of the external seminal vesicle, i.e., near the anterior margin of the ovary (Fig. 5C); cirrus-sac situated more posterior between the ovarian wings (Fig. 5C); parasite of *Moxostoma anisurum* and *M. erythrurum*..... *B. longicollum* n. sp.
- 3a. Width of the cirrus-sac <35% of the body width at the same level (Fig. 2C); cirrus-sac does not extend anterior to the ovarian wings, situated more posteriorly between the ovarian wings (Fig. 2C); parasite of *M. collapsum* *B. isaureae* n. sp.
- 3b. Width of the cirrus-sac >35% of the body width at the same level (Fig. 7C); cirrus sac situated more anteriorly between the ovarian wings (Fig. 7C); parasite of *Minytrema melanops* *B. overstreeti* n. sp.

Discussion

Molecular and morphological evaluation of newly collected material of *Biacetabulum* tapeworms with an extraordinarily long neck from several catostomids, especially redhorses, in North America provided evidence of the existence of a species complex in this genus (Fig. 12). It is possible that this species complex represents an example of recent speciation of tapeworms in different, partly congeneric fish hosts, as documented by subtle, but consistent morphological differences between tapeworms of individual genetic lineages/morphotypes. A similar case of likely recent speciation in different fish hosts has been documented only in the caryophyllidean genus *Caryophyllaeus* Gmelin, 1790, parasites of cyprinids in Eurasia (see Barčák *et al.*, 2014, 2017; Bazsalovicsová *et al.*, 2014; Hanzelová *et al.*, 2015). It is also possible that the small genetic and morphological changes in this species complex reflect slow diversification accompanied by host-shifting and geographical range expansion.

The existence of a species complex has also been revealed by molecular data in *Paracaryophyllaeus* Kulakovskaya, 1961, parasites of Eurasian loaches. However, no clear host-related pattern of speciation was detected in this group of Eurasian caryophyllideans by Scholz *et al.* (2014). Future phylogenetic and biogeographical analyses using a more comprehensive data set may provide evidence of the diversification mode in this long-necked *Biacetabulum* species complex.

The present data provide another evidence that the actual species diversity of North American caryophyllideans is higher than previously thought. *Biacetabulum* contained 10 species recognised by Scholz and Oros (2017), of which 9 occur in the Nearctic region (the taxonomy of *B. tandoni* Johnston et Muirhead, 1950 from Australia remains questionable). Based on the present study, the total number of species of the genus increases to 14, but the actual species richness of *Biacetabulum* is undoubtedly much higher, as indicated by a recent molecular phylogenetic study (Scholz *et al.*, 2021) as well as our unpublished results from caryophyllideans recently collected in the southern USA. For example, tapeworms found in grey redhorse, *M. congestum* (Baird and Girard, 1854) from the Pedernales River, Texas, and those from shorthead redhorse, *M. macrolepidotum* (Lesueur) in the Cedar River, Wisconsin, found by the present authors may also belong to yet undescribed species of *Biacetabulum* (unpublished data).



Fig. 12. Map of the distribution of the newly described species of *Biacetabulum*. Circle – *B. longicollum* n. sp.; triangle – *B. isaureae* n. sp.; square – *B. overstreeti* n. sp.; empty square – *B. hypentelii* n. sp.

The high diversity of scolex types in Nearctic caryophyllideans (Mackiewicz, 1972) was highlighted in a recent study by Oros *et al.* (2020) that also drew attention to the relatively high variation in scolex shape among some congeneric species, including those of *Biacetabulum*. In contrast, the present study of four species of the long-necked *Biacetabulum*-species complex from disparate hosts revealed a rather uniform scolex morphology. The newly described species of the long-necked *Biacetabulum*-species complex exhibit a relatively high/strict (oioxenous or stenoxenous) host specificity because they occur in a single host species or in two (*B. longicollum*) congeneric species of definitive hosts. However, new material is needed to confirm this strict host specificity.

The present study provides yet more evidence for the high, yet poorly known species diversity of caryophyllidean tapeworms in North America. It also points out the need to apply methods of integrative taxonomy to properly collected and processed specimens of these fish parasites. It is obvious that future studies should critically scrutinise existing information – in the form of literature and museum depositions – on host associations and distribution of caryophyllideans, which represent a dominant component of communities of intestinal helminths in catostomid fishes in North America (Kuchta *et al.*, 2020).

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Author contribution. MO and AC collected material. TS and MO designed the study. DU, MO, and TS conceived morphological study (DU made line drawings). OK conceived molecular phylogenetic analyses. TS drafted the text and all other authors contributed to writing the manuscript.

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Ethical standards. Fish examined for parasite were euthanised humanely, following the Ethical Standards of individual research institutions.

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